DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms

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Disrupted-In-Schizophrenia-1 (*DISC1*) is a promising susceptibility gene for major mental illness, but the mechanism of the clinical association is unknown. We searched for *DISC1* transcripts in adult and fetal human brain and tested whether their expression is altered in patients with schizophrenia and is associated with genetic variation in *DISC1*. Many alternatively spliced transcripts were identified, including groups lacking exon 3 (Δ3), exons 7 and 8 (Δ7Δ8), an exon 3 insertion variant (extra short variant-1, Esv1), and intergenic splicing between *TSNAX* and *DISC1*. Isoforms Δ7Δ8, Esv1, and Δ3, which encode truncated DISC1 proteins, were expressed more abundantly during fetal development than during postnatal ages, and their expression was higher in the hippocampus of patients with schizophrenia. Schizophrenia risk-associated polymorphisms [non-synonymous SNPs rs821616 (Cys704Ser) and rs6675281 (Leu607Phe), and rs821597] were associated with the expression of Δ3 and Δ7Δ8. Moreover, the same allele at rs6675281, which predicted higher expression of these transcripts in the hippocampus, was associated with higher expression of *DISC1*Δ7Δ8 in lymphoblasts in an independent sample. Our results implicate a molecular mechanism of genetic risk associated with *DISC1* involving specific alterations in gene processing.

alternative splicing | genetic | hippocampus | psychiatric illness

Schizophrenia is a common mental disorder with a lifetime prevalence of ≈ 1% (1). It has been assumed, on the basis of evidence from twin, family, and adoption studies, that genetic factors play a strong etiologic role in schizophrenia (2, 3). The genetic predisposition is likely to be determined by a complex network of interactions between genes and environmental risk factors (4).

The Disrupted-In-Schizophrenia-1 (*DISC1*) gene was identified as a potential susceptibility gene for mental disorders based on studies of a chromosomal translocation found in a large Scottish family that had a high frequency of schizophrenia and other psychiatric disorders, including bipolar disorder and major depression (5–7). Although the translocation identified in the Scottish family has not been observed in any other families, independent support for involvement of *DISC1* in the etiology of mental illness has come from a number of genetic linkage and association studies in diverse populations (8–25). Although the specific SNPs, alleles, and haplotypes have varied across these studies, raising issues of allelic and populations heterogeneity, the overall evidence implicates *DISC1* as a promising candidate susceptibility gene for schizophrenia. However, the mechanism by which *DISC1* contributes to the pathophysiology of schizophrenia remains unknown. Although reduced expression of *DISC1* mRNA was found in lymphoblastoid cell lines of family members with the translocation (26) and in bipolar disorder patients with a putative risk-associated haplotype (27), no changes were detected in the brain tissue of unrelated patients with schizophrenia or in individuals carrying risk-associated genotypes (28–30). The evidence from a variety of studies in cell and animal models as well as in human brain tissue suggests that *DISC1* mRNA and/or protein processing is complex, and it therefore is plausible that changes in gene processing occur in patients who have major mental illness (28, 31–36).

In this study, we characterize *DISC1* mRNA processing in adult and fetal human brain and in lymphoblasts. We show that transcripts encoding truncated DISC1 proteins in transfected HEK293 cells are expressed at significantly higher levels in the cerebral cortex during normal human fetal development than later in life, are enriched in the hippocampus of patients with schizophrenia, and are related to the previously identified risk-associated polymorphisms (20–21, 37–40). Our data suggest that a molecular mechanism by which variation in *DISC1* impacts risk for psychiatric illness involves specific alternative gene processing.

Results

Identification of *DISC1* Splice Variants. Portions of human *DISC1* mRNA expressed in postmortem human hippocampus were amplified using selected combinations of primers to search for possible alternatively spliced transcript variants (Fig. S1; Table S1). We observed 5 fragments of unpredicted size (Fig. S2). Direct sequencing of these fragments revealed that they were skipping exon 3 (Δ3) and exons 7 and 8 (Δ7Δ8). Both products, Δ3 and Δ7Δ8, were less abundant than products with exons 3, 7 and 8 present (Fig. S2). The remaining 3 fragments suggested the presence of a group of transcripts terminating with a unique exon 3a of the DISC1 isoform Es, which was registered as an alternative final exon of Es (NM_001012958.1), but containing exon 3. One of them (Esv1) contained exon 3 spliced to the unique final exon 3a. The other 2 fragments (Esv2 and Esv3) contained either an exon of 77 nucleotides (E77) or an exon of 133 nucleotides (E133). Sequences of these 2 exons were identical to the *DISC1* genomic sequence (NT_004559.13). Splice boundaries of the exons conformed to GT-AG consensus. We also amplified and confirmed portions of the DISC1 isoforms Lv (NM_001012957.1) and S (NM_001012959.1) (Fig. S2), previously reported in human tissue (32–34). Furthermore, all fragments also were detected in adult dorsolateral prefrontal cortex tissue, fetal frontal cortical tissue, and B lymphoblasts.

To identify 5′ and 3′ ends of putative transcripts, multiple 5′ RACE and 3′ RACE reactions were carried out using fetal brain RNA with *DISC1* gene-specific antisense primers located in exon 3a, 4, and exon 9 and sense primers binding to exon 2 and exon 6 (Table S2). Sequencing of the 5′ RACE products revealed the presence of 2 alternative first exons of *DISC1*, exon 1 of a

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reported *DISC1* transcript (NM_018662.2) and exon 1 of the Translin-associated factor X (*TSNAX*, *TRAX*) gene (Fig. S3). This 5′ RACE product initiated at *TSNAX* exon 1 represents an ORF through to *TSNAX* exon 4, which then is spliced directly to *DISC1* exon 2. This type of transcript has been reported previously as one of *TSNAX/DISC1* fusion transcripts resulting from intergenic splicing ("*TRAX* exon 4 + *DISC1* exon 2" in ref. 41). Furthermore, using 5′ RACE, we confirmed the existence of products Δ3, Δ7Δ8, and Esv1, starting with *DISC1* exon 1.

Characterization of the 3′ RACE products revealed the presence of 10 alternative termination exons: exon 13, exon 9a, and exon 3a corresponding to the termination exons of *DISC1* isoform L (also common to Lv), S, and Es, respectively, as well as termination exons 3, 4, 6, 9, 9b, 10, and 11, Fig. S3. Using long-range PCR with primers listed in Table S3, followed by sequencing of the products, we identified 54 full-length *DISC1* transcripts, including the transcripts numbered 3–21 and 23–54 in Fig. 1 with consecutive GenBank accession numbers FJ804174-FJ804192 and FJ804193-FJ804224 as well as the known transcripts L, Lv, and S. All exons that we identified in this study were identical to the *DISC1* genomic sequence (NT_004559.13) and conformed to the consensus donor and acceptor sequences. Intergenic *TSNAX/DISC1* transcripts are all predicted not be translated into fusion proteins, because the ORFs starting from *TSNAX* exon 1 would be discontinued by a premature stop codon.

We transfected HEK293 cells with Myc-fusion constructs containing the following *DISC1* variants: L, Lv, S, Esv1 (#43 FJ804213), LΔ7Δ8 (#32 FJ804202 and #17 FJ804188), LΔ3 (#42 FJ804212 and #3 FJ804174), and TΔ5Δ6-B-DΔ1Δ7 (#47 FJ804217). Using Western blotting with an anti-Myc antibody, we determined that 7 of these variants, but not LΔ3 (#3, FJ804174) and TΔ5Δ6-B-DΔ1Δ7 (#47, FJ804217), which have premature stop codons, are translated into proteins, as indicated by single bands of approximately predicted sizes (Fig. S4*A*).

Expression of *DISC1* Splice Variants in Schizophrenia. We measured mRNA expression levels of *DISC1* transcripts with deletions of exon 3 and exons 7 and 8 (Δ3, Δ7Δ8) and an isoform Esv1 as well as known transcripts of *DISC1* and *TSNAX* in the hippocampus by quantitative real-time PCR (Table S4). All data are normalized to a geometric mean of housekeeping genes (*ACTB*, *GUSB*, *B2M*) (Table S4) and co-varied by age at death, postmortem interval, pH, RNA integrity number, sex, and smoking status.

We found that the expression of Δ7Δ8 mRNA was significantly increased in the hippocampus of patients with schizophrenia as compared with controls [by 26%, ANCOVA: F (1, 79) = 5.1, P = 0.027] (Fig. 2*A*; see Table S5 for subject demographics). Expression of Esv1 also was significantly higher in patients with schizophrenia [by 22%, F (1, 83) = 6.4, P = 0.013]. In addition, Δ3 and Lv transcripts tended to be more highly expressed in schizophrenia: Δ3 by 20% [F (1, 84) = 2.8, P = 0.096], and Lv by 10% [F (1, 84) = 2.8, P = 0.097]. Furthermore, the relative expression levels of these short transcripts and Lv calculated as ratios to the expression of a majority of *DISC1* transcripts (DISC1_All) were significantly increased in schizophrenia (by ≈30%−40%, F values > 4.0, P < 0.01). On the other hand, expression of DISC1_All, the expression of full-length DISC1 (L), and the expression of *DISC1* transcripts with exons 3 and 4 present (*DISC1 3/4*) were not different in the hippocampus of patients with schizophrenia as compared with controls (all F values < 1.0, P > 0.5). There also were no differences in the expression of any *TSNAX* transcripts: *TSNAX 5/6* that recognized only *TSNAX* transcripts, *TSNAX 3/4* that recognized *TSNAX* transcripts and a part of *TSNAX/DISC1* fusion transcripts, and *TSNAX 1/2* that detected expression levels of all *TSNAX* and *TSNAX/DISC1* fusion transcripts (all F values < 1.0, P > 0.5) (Fig. 2*A*). There were no significant effects of history of substance abuse, age at the onset of the disease, age at first hospitalization, duration of illness, or estimated daily, life-time, and last dose of neuroleptics on the expression of any of the *DISC1* transcripts in patients with schizophrenia.

Effects of *DISC1* Genotype on *DISC1* Splice Variant Expression. We examined the effects of 12 previously assessed *DISC1* SNPs (21) on the mRNA expression levels of *DISC1* splice variants in the same cohort (Fig. S5). All data were co-varied by age at death, pH, RNA integrity number, sex, and smoking status. The mRNA expression of Δ3 was significantly associated with an intronic SNP rs821597 [F (2, 79) = 4.10, P = 0.02] (Fig. 2*C*). In particular, individuals homozygous for the A allele at rs821597 had significantly higher expression than heterozygous G/A individuals (P < 0.01). There was no significant effect of race (F = 0.09, P > 0.5) or race by genotype interaction (F = 1.8, P = 0.16). Separate analysis in African-Americans (performed because the allelic frequencies differed between the racial groups) showed that rs821597 was significantly associated with the Δ3 expression [F (2, 50) = 4.34, P = 0.02], and the effect was allele dose dependent; that is, homozygous A/A individuals had increased Δ3 expression as compared with the G allele carriers (G/G and G/A) (P < 0.05). There were no significant effects in the white cohort alone (P > 0.5), but this analysis clearly is underpowered.

Furthermore, we found significant effects of 2 coding SNPs, rs6675281 (Leu/Phe) and rs821616 (Ser/Cys), on the expression of Δ7Δ8 mRNA. The T (Phe) carriers (the 3 T/T subjects combined and the C/T subjects) showed significantly higher expression than C/C (Leu/Leu) individuals [by 22%, F (1, 81) = 6.5, P = 0.01] (Fig. 2*C*). There were no significant interactions between diagnosis and genotype or between race and genotype (F < 0.05, P > 0.8). For rs821616, there was a main effect of genotype in the whole sample [F (2, 79) = 4.07, P = 0.02] (Fig. 2*C*), a trend for a race effect (F = 2.9, P = 0.09), and a trend for a significant race by genotype interaction (F = 2.4, P = 0.09). Individuals homozygous for the T (Cys) allele had significantly increased expression of Δ7Δ8 mRNA compared with individuals carrying the A (Ser) allele (P < 0.01). This association was significant and strong in the African-American sample, in whichΔ7Δ8 expression was significantly higher in the homozygous T/T (Cys/Cys) individuals than in the homozygous A/A (Ser/Ser) (P = 0.003) or the heterozygous A/T individuals (Ser/Cys) (P = 0.001) but was not significant in the white sample, again perhaps because of lack of power. No other SNP examined in this study showed any effect on the expression of Δ3 and Δ7Δ8 (all P values > 0.05), and none was associated with the expression of other transcripts. Thus, 2 unlinked coding SNPs associated in several studies with risk for major psychiatric illness show similar effects on the expression of transcripts up-regulated in schizophrenic brain tissue, and for both SNPs the risk-associated allele predicts increased expression of these transcripts.

To confirm the observations of these SNP effects in a tissue that is not subject to the effects of confounding factors, such as medication and other pre- and postmortem changes, we measured expression of Δ3 and Δ7Δ8 in cultured B lymphoblasts from an independent group of only white individuals (n = 56, mean age 34 ± 11 years, female:male ratio 1:1). In this group, we did not find the effect of rs821597 on the expression of Δ3 (P > 0.05), which in the hippocampus was seen mainly in the African-American sample. For rs6675281, however, in agreement with the data in the hippocampus, Δ7Δ8 expression was significantly higher in the T (Phe) carriers than in the C/C (Leu/Leu) homozygotes (by 59%, t = 2.2, P = 0.03) (Fig. S4*B*). Although there was no statistically significant effect of genotype on Δ7Δ8 expression for rs821616, the single subject with the T/T (Cys/Cys) genotype had the highest expression of all subjects (Fig. S4*B*).

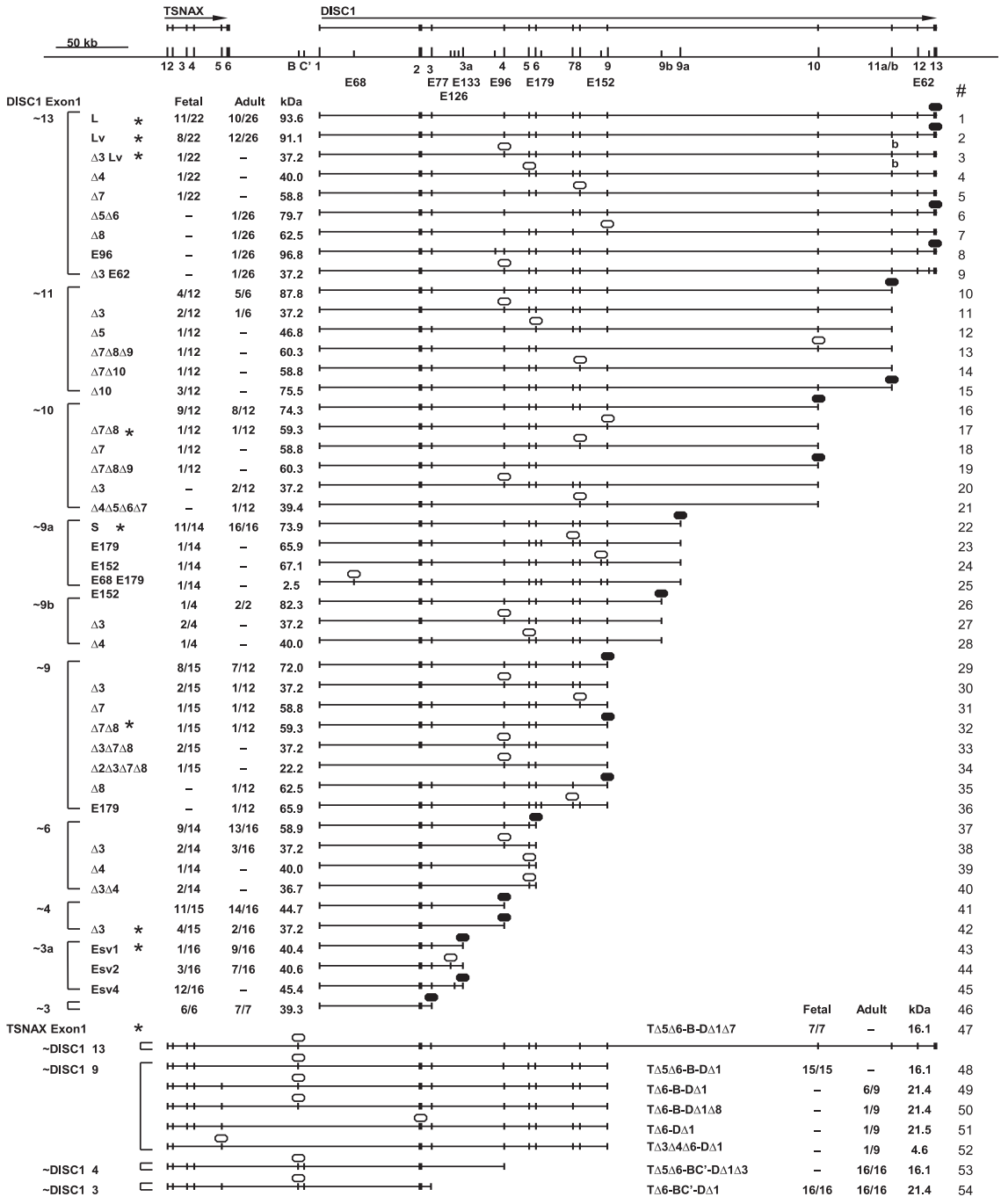

Fig. 1. Schematic representation of the genomic organization of alternative splice variants of the human *DISC1* gene. The continuous line represents the genomic sequence, on which known exons of *DISC1* and *TSNAX* are shown as rectangles and are numbered. Exons C′ located between the *TSNAX* gene and the *DISC1* gene, E68 in intron 2, E77, E126, E133, and E96 in intron 3, E179 in intron 6, E152 in intron 8, 9b in intron 9, and E62 in intron 12 and the exon previously identified as *TSNAX*/*DISC1* exon B (6) are shown as smaller rectangles. For each transcript (numbered on the right from 1–54), the number of clones in which the transcript has been identified is shown per a total number of clones examined in each cloning experiment of end-to-end PCR products. Ovals indicate the locations of stop codons for the ORF starting with either *DISC1* exon 1 or *TSNAX* exon 1. Open ovals indicate premature termination codons (PTC); closed ovals indicate normal stop codons. The predicted molecular weight of each protein isoform was calculated using the Compute pI/Mw tool (Swiss Institute of Biotechnology). Variants used in transfection experiments are marked with asterisks.

Expression of *DISC1* Transcripts During the Life Span. To investigate the developmental profile of expression of the transcripts, we used samples from the prefrontal cortex of a different cohort of normal controls spanning almost the entire human life span, from the 14th through 20th gestational week and from birth through old age (78 years of age). (See Table S5 for subject demographics.) Expression of Δ3, Δ7Δ8, and Esv1 transcripts were much higher during fetal development, dropped around birth, and remained very low throughout postnatal life (Figs. S4 *C* and *D* and S6*A*). Long transcripts (with exons 12 and 13 present) also were higher before birth, but the difference between fetal and postnatal expression was much less pronounced than for the short transcripts (Fig. S6*B*). We next tested whether expression of short transcripts was related to genotype during the

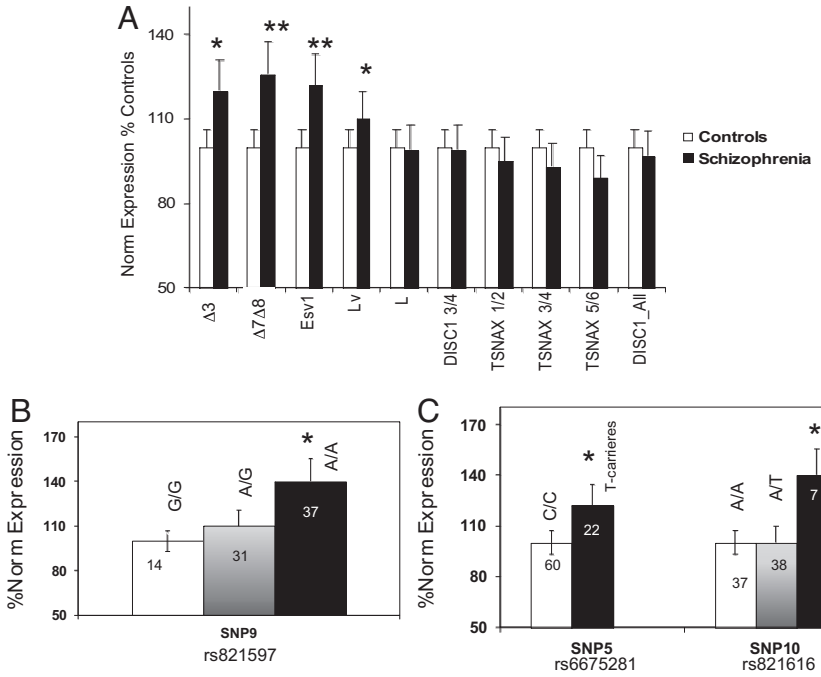

Fig. 2. (*A*) Expression of *DISC1* and *TSNAX* transcripts in the hippocampus of patients with schizophrenia and normal controls (means ± SE). Abbreviations: Δ3, deletion of exon 3; Δ7Δ8, deletion of exons 7 and 8; *DISC 3/4*, transcripts with exons 3 and 4 present; DISC_All, transcripts with exon 2 present (a majority of all transcripts); Esv1, extra short variant; L, full length variant; Lv, long variant; *TSNAX 1/2*, all *TSNAX* and *TSNAX/DISC1* fusion transcripts; *TSNAX 3/4*, all *TSNAX* transcripts and a part of *TSNAX/DISC1* fusion transcripts; *TSNAX 5/6*, only *TSNAX* transcripts. **, Significantly different from controls, $P < 0.05$; *, different from controls, $P < 0.10$. (*B*) Association of rs821597 (SNP9) and Δ3 mRNA expression in the hippocampus. (*C*) Associations between rs6675281 (SNP5) and rs821616 (SNP10) and Δ7Δ8 mRNA expression in the hippocampus. *, Significantly different from other groups, $P < 0.05$. Error bars in *A*–*C* are SD of the mean.

fetal period, when there was the greatest change in the expression with age. Indeed, G/A subjects at rs821597 showed a predicted reduction of Δ3 with age during the fetal period ($R = -0.59$, $P = 0.009$) (Fig. S6*C*), but A/A homozygotes did not show such downregulation with age ($R = 0.25$, $P = 0.2$) (Fig. S6*D*). The slopes of the 2 regression lines differed significantly ($P < 0.05$). Similar differences in the pattern of expression of Δ7Δ8 over fetal age were seen between individuals carrying either the A or the T allele at rs821616, but the differences were not significant ($P > 0.05$); that is, the expression of Δ7Δ8 in subjects carrying the A (Ser) allele was diminished predictably with increasing fetal age ($R = -0.2$), whereas homozygotes for the T (Cys) allele, associated previously with high Δ7Δ8 expression, did not show such downregulation ($R = 0.14$).

Discussion

We report a detailed characterization of alternative splice variants of the *DISC1* gene in human brain and show that it produces more than 50 splice variants in the brain. We also have examined the developmental profiles of expression of *DISC1* mRNA variants and the effects of schizophrenia diagnosis and risk-associated genotypes on expression. We show that Δ7Δ8 and Esv1 were more abundant in the hippocampus of patients with schizophrenia and that Δ3 and Lv also tended to be increased in patients with schizophrenia, whereas overall *DISC1* expression levels were not changed in patients. Furthermore, Δ3 and Δ7Δ8 expression levels were significantly associated with risk-related genetic variations in the *DISC1* gene. Finally, short transcripts were expressed at much higher levels in fetal brains than in postnatal brains, suggesting that they may play important roles in early brain development. Our data also suggest that the developmental trajectories of these transcripts are associated with genotype; genotype that predicted abnormally high expression of a *DISC1* transcript also was associated with lack of normal downregulation of expression during fetal development.

Splicing of *DISC1* mRNA proved to be complex and highly variable but perhaps not unusual. Several genome-wide studies estimate that 40% to 75% of human genes have alternative splice forms (42–46). Alternative splicing plays a major role in modulating gene function by increasing the diversity of expressed mRNA transcripts (44, 47) and is considered to be particularly prevalent in human brain, which has a greater number of splice variants than any other organ (48, 49). Computational and experimental evidence indicates that approximately 1/3 of alternatively spliced transcripts in the human contain a premature termination codon (PTC) and thus may be degraded, but not necessarily completely eliminated, by the nonsense-mediated mRNA decay (NMD) (50–52). Most of the *DISC1* transcripts that we identified in this study contained a PTC. We have shown that at least some transcripts that are expressed at higher levels in schizophrenia and have normal stop codons or a PTC make proteins of predicted sizes in HEK293 cells. Whether these variants can be translated into proteins in human brain cells and whether they are functional remains to be determined.

Short transcripts may be associated with schizophrenia in a dominant-negative fashion, because the putative truncated proteins lack the C terminus predicted to mediate protein−protein interactions with a large number of proteins, including nuclear distribution E-like (NDEL1, NUDEL), lissencephaly 1 (LIS1), and elongation protein zeta-1 (FEZ1) (33, 41, 53–59). Indeed, there is ample evidence from cell and animal models that overexpression of C-terminally truncated DISC1 leads to aberrant phenotypes. Transfection of cells with truncated DISC1 isoforms results in aberrant intracellular localization of DISC1, disruption of interactions with other proteins, including NDEL1, LIS1, dynactin, dynein, and PDE4B, aberrant mitochondrial function, disrupted microtubular network, and inhibition of neurite outgrowth (26, 53, 55, 57, 59, 60). Mice, in which C-terminally truncated forms of DISC1 were overexpressed on a background of endogenous mouse DISC1, show brain morphological changes, cytoarchitectural anomalies in the hippocampus and cortex, diminished neurite outgrowth, and various behavioral abnormalities reminiscent of aspects of human mental illness (61–63).

We found evidence for an association between Δ3 and Δ7Δ8 expression and genetic variations in the *DISC1* gene. Individuals homozygous for the A allele at rs821597or the T (Cys) allele at rs821616 and carriers of the T (Phe) allele at rs6675281 were found to have greater expression of Δ3 and Δ7Δ8 transcripts. Moreover, these genotypes predicted unique patterns of expression of short

transcripts during fetal development. These data suggest that even normal individuals with risk-associated alleles in *DISC1* show different age-related regulation of short transcripts than carriers of non-risk alleles. Given the evidence that schizophrenia is a neurodevelopmental disorder with a strong genetic component, it seems plausible that the risk-associated SNPs exert their pathological effects by affecting expression of alternative transcripts that are predominantly expressed early in human brain development.

We have searched Polyphen (http://genetics.bwh.harvard.edu/pph/) (64) and RegRNA (http://regrna.mbc.nctu.edu.tw/) (65) to predict *in silico* functional consequences of risk-associated SNPs. RegRNA predicts that rs821597 contains a regulatory motif for an intron enhancer that may be involved in splicing. According to Polyphen, substitutions in both the coding SNPs, Ser/Cys (rs821616) and Leu/Phe (rs6675281), are predicted to be "benign" based on the position-specific independent counts. Cys-704 *DISC1*, however, was shown to be associated with relatively lower biological activity on ERK signaling, reduced brain gray matter volume, and an increased risk for major depressive disorder as compared with Ser (37). Phe-607 is associated with poorer co-localization with pericentriolar material 1 and reduced noradrenaline release as compared with Leu-607 in transfected SH-SY5Y cells (66). Moreover, this SNP appears to contain a regulatory motif that would alter binding of an exonic splicing enhancer, SRp20, on the forward strand and an exon enhancer, hnRNPG, on the reverse strand. This latter binding site may be relevant for the expression of *DISC2*, which is transcribed in the opposite direction to *DISC1*. To test this possibility, we have conducted a preliminary analysis of prefrontal cortical samples from normal controls and found that, indeed, *DISC2* mRNA expression tends to be predicted by rs6675281 genotype, raising the possibility that *DISC2* may be involved in the regulation of *DISC1* splice variants ($F = 2.0$; $P = 0.11$; $n = 148$). We currently are extending this study to a larger cohort.

In the previous study from our group, there were significant associations of the A (Ser) allele at rs821616 and a 3-marker haplotype (C allele at rs7546310 - G allele at rs821597 - A (Ser) allele at rs821616) with increased risk for schizophrenia and a reduction of hippocampal volume in a white population (21). The inconsistencies between the risk alleles in the previous and the current study may relate to genetic and allelic heterogeneity in the white and African-American populations. In the present study, the cohort consisted predominantly of African-American subjects. Indeed, no effects of the 2 SNPs on Δ3 and Δ7Δ8 expression were detected in the white cohort, although the statistical power may have been inadequate because of small numbers. Similarly, in a Chinese Han population, the A allele at rs821597 and the T (Cys) allele at rs821616 were reported as risk alleles for schizophrenia (22), and in a Japanese population the Cys-704 allele was associated with an increased risk of developing major depressive disorder (37). Thus, phenotypic effects of these genetic variations may be different among ethnic groups. Alternatively, it is possible that these SNPs are mere genetic markers for unknown functional variants that are more directly associated with schizophrenia. Notwithstanding these uncertainties, it is remarkable that 2 unlinked risk-associated coding SNPs in *DISC1*, which have not been found to be positive together in any particular study, are each independently associated with the same pattern of expression of abundant fetal splice variants. This finding may implicate a molecular mechanism of allelic heterogeneity in *DISC1*. As in classic Mendelian genetic disorders, such as cystic fibrosis, in which multiple mutations are associated with a common phenotype because they affect the same protein function, a common effect on gene processing suggests that multiple regulatory variants in *DISC1* may be associated with a common phenotype.

A serious limitation of our study is that we did not perform corrections for multiple testing in our analysis. We believe that such correction would be unduly conservative, given that we focused on comparisons that were positive in the previous studies and that we predicted that the expression of short but not long transcripts would be altered. Moreover, we have replicated an association of Δ7Δ8 with a genotype in an independent cohort in lymphoblasts. Nonetheless, our finding regarding the association of *DISC1* splicing with the 3 SNPs requires further independent replications.

Conclusions

Alternative splicing of the human *DISC1* gene in brain is complex and yields a large number of transcripts. We demonstrated here that short transcripts, some of which are predicted to be degraded by the NMD pathway and others that may produce functionally aberrant and truncated proteins, are more highly expressed during fetal development than later in life, are more highly expressed in the brains of patients with schizophrenia than in control brains, and are associated with genetic variations in the *DISC1* gene. The present study suggests that at least one molecular mechanism of the genetic association of *DISC1* with major psychiatric disorder is the regulation of expression of specific alternative splice forms of this gene, especially during fetal brain development.

Materials and Methods

Human Tissue—Subjects and RNA Extraction. Postmortem brains were collected at the Clinical Brain Disorders Branch (CBDB), National Institute of Mental Health (NIMH) with informed consent from the legal next of kin under NIMH protocol 90-M-0142 and at the Brain and Tissue Bank for Developmental Disorders of the National Institute of Child Health and Human Development under contracts NO1-HD-4–3368 and NO1-HD-4–3383 and were processed as described in detail elsewhere (67) (Table S5 and *SI Materials and Methods*).

Transfection and Immunoblotting. Human embryonic kidney cell line (HEK293) was cultured using standard methods (see *SI Materials and Methods* for details).

PCR Reactions. Total RNA was extracted from postmortem hippocampal tissues of 8 adult normal controls, 8 fetal normal controls, and 8 lymphoblast cell lines and was reverse transcribed (see *SI Materials and Methods* for details).

Quantitative Real-Time PCR. Expression of mRNA was measured using an ABI Prism 7900 sequence detection system with 384-well format (Applied Biosystems) by a standard curve method as described previously (67), using assays-by-design and custom-made assays designed using PRIMER EXPRESS software (Applied Biosystems) (Table S4). The expression data were normalized to a geometric mean of 3 housekeeping genes, *ACTB*, *GUSB*, and *B2M*.

Genotyping. DNA was extracted from cerebellar brain tissues using PUREGENE (Gentra Systems). Genotyping of 12 SNPs was performed using the Taqman 5′ exonuclease allelic discrimination assay as described previously (21) (Fig. S5). Genotype reproducibility was > 99%.

Statistical Analyses. Statistical analyses were conducted using STATISTICA version 7.1 (StatSoft Inc.). Comparisons between groups were made using ANCOVA for each mRNA with diagnosis, genotype, and race as independent variables and sex, pH, postmortem interval, age, and RNA quality as covariates.

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